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# Urinary Ethyl Glucuronide Can Be Used as a Biomarker of Habitual Alcohol Consumption in the General Population

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## ABSTRACT

**Background:** Alcohol consumption is a frequently studied risk factor for chronic diseases, but many studies are hampered by self-report of alcohol consumption. The urinary metabolite ethyl glucuronide (EtG), reflecting alcohol consumption during the past 72 h, is a promising objective marker, but population data are lacking.

**Objective:** The objective of this study was to assess the reliability of EtG as a marker for habitual alcohol consumption compared with self-report and other biomarkers in the general population.

**Methods:** Among 6211 participants in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort, EtG concentrations were measured in 24-h urine samples. EtG was considered positive when concentrations were  $\geq 100$  ng/mL. Habitual alcohol consumption was self-reported by questionnaire (categories: no/almost never, 1–4 units per month, 2–7 units per week, 1–3 units per day or  $\geq 4$  units per day). Plasma HDL cholesterol concentration, erythrocyte mean corpuscular volume (MCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and  $\gamma$ -glutamyltransferase (GGT) were determined as indirect biomarkers of alcohol consumption. Sensitivity, specificity, positive and negative predictive value, and proportions of agreement between reported consumption and EtG were calculated. To test the agreement of EtG concentration and alcohol consumption in categories, linear regression analysis was performed. In addition, the association between EtG concentrations and indirect biomarkers was analyzed.

**Results:** Mean age was 53.7 y, and 52.9% of participants men. Of the self-reported abstainers, 92.3% had an EtG concentration  $< 100$  ng/mL. Sensitivity was 66.3%, positive predictive value was 96.3%, and negative predictive value was 47.4%. The proportion of positive agreement was 78.5%, and the proportion of negative agreement was 62.7%. EtG concentrations were linearly associated with higher categories of alcohol consumption ( $P$ -trend  $< 0.001$ ), adjusted for age, sex, and renal function. EtG was positively related to MCV, HDL cholesterol, and GGT but not to AST and ALT concentrations.

**Conclusions:** This study shows that urinary EtG is in reasonable agreement with self-reported alcohol consumption and therefore can be used as an objective marker of habitual alcohol consumption in the general population. *J Nutr* 2019;149:2199–2205.

**Keywords:** ethyl glucuronide, habitual alcohol consumption, self-report, biomarker, general population

## Introduction

Alcohol consumption is frequently studied as a risk factor for chronic diseases (1–3). The vast majority of these studies are observational and rely on self-reported alcohol consumption. Self-report has proven to be a relatively unreliable source

of information, often leading to underreporting of alcohol consumption (4). There obviously is a need for objective markers of alcohol consumption that can be easily assessed.

The determination of ethanol in blood, exhaled breath, or urine has a very limited detection window and only expresses alcohol consumption during the past few hours (5). Indirect

biomarkers, such as liver enzymes, are usually markers of chronic excessive alcohol consumption (6). These markers do not directly represent metabolites of alcohol but merely express the influence of alcohol on various organs. Also, other factors are known to influence these markers, including nonalcoholic liver diseases, which cause a rise in liver enzymes (7). In general, indirect biomarkers are not specific enough to be suitable markers for habitual alcohol consumption.

During the past several decades, new markers of alcohol consumption have been identified (7, 8). A promising marker is the urinary metabolite ethyl glucuronide (EtG), a direct marker of alcohol consumption. It is the small part (<0.1%) of ethanol that is not oxidized by the liver but is excreted in urine as EtG after conjugation with glucuronic acid. After complete elimination of ethanol, EtG has a half-life of 2–3 h (9). Therefore, EtG is detectable in urine for a longer time period than ethanol, with a window of detection of ~72 h (10). Many studies have used EtG as a marker in abstinence programs in alcohol-dependent persons (11–16). In these often controlled studies, EtG has been validated as a reliable marker to detect short-term alcohol consumption. Less frequently, EtG has been studied as a potential marker for habitual alcohol consumption in the general population. To date, 1 study has assessed the value of EtG as a potential marker of habitual, nonabusive alcohol consumption in a population-based study (17) by comparing urinary EtG concentrations in 175 male workers to other known alcohol markers. This study showed that higher urinary EtG concentrations were associated with higher concentrations of other biochemical markers of alcohol consumption. The relation between urinary EtG and self-reported alcohol consumption remains to be investigated.

In the current study, we investigated the association between self-reported alcohol consumption and EtG as a marker of habitual alcohol consumption in a large general population-based cohort. Furthermore, we compared EtG concentrations with other known biomarkers of alcohol consumption to explore the potential use of EtG as a new objective marker in population-based research.

## Methods

### Study population

The Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort is a Dutch longitudinal cohort drawn from the general population in the city of Groningen in 1997, originally established to monitor the long-term development of cardiovascular and renal diseases in participants with microalbuminuria. Details of this study have been published elsewhere (18). In summary, all inhabitants aged 28–75 y ( $n = 85,421$ ) were asked to provide a morning urine sample and to complete a short questionnaire. In total, 40,856 (47.8%) participants responded. Subjects with a urinary albumin concentration >10 mg/L were invited for further participation. After exclusion of pregnant

women and participants using insulin, 6000 participants with a urinary albumin concentration >10 mg/L were included. In addition, a random sample of 2592 participants without microalbuminuria was included. The total number of participants was 8592. The PREVEND study was conducted in accordance with the Declaration of Helsinki guidelines and was approved by the Medical Ethics committee of the University Medical Center Groningen. All participants gave written informed consent. During the study period (1997–2013), participants attended 5 subsequent screening rounds.

In the current study, we included participants who attended the second screening round ( $n = 6894$ ), which lasted from April 2001 until December 2003, because urinary EtG concentrations were measured in urine samples that were collected during this period. We excluded participants without EtG measurements ( $n = 60$ ) or for whom data on self-reported alcohol consumption were missing ( $n = 64$ ). Moreover, we excluded participants when urinary analysis (urinary leukocyte measurements done by Nephur-test + leuco sticks; Boehringer Mannheim) showed evidence of a urinary tract infection, defined as a test result >“2+ leukocytes” ( $n = 363$ ) or >“3+ erythrocytes” ( $n = 196$ ). Previous research has shown that bacterial contamination can influence EtG concentrations, which can lead to both false-positive and false-negative results (8, 19). The analytic sample included 6211 participants.

### Biomarkers of alcohol consumption

#### Ethyl glucuronide.

Participants were asked to collect two 24-h urine samples maximal 4 d before the second visit after thorough oral and written instruction. In this instruction, the participants were asked to avoid heavy exercise as much as possible during the urine collection and to postpone the urine collection in case of urinary tract infection, menstruation, or fever. Participants stored the samples temporarily at home at a temperature of 4°C prior to the visit. After handing in, aliquots of these urine specimens were stored at –20°C. EtG concentrations were measured in the second 24-h urine sample using the Thermo Scientific DRI EtG assay, which is commonly used as a screening test for EtG. It has a detection limit of 100 ng/mL. Previously, this assay has shown overall good agreement ( $r^2 = 0.931$ ) with established LC-MS methods in detecting EtG. Moreover, agreement was shown to be equally good ( $r^2 = 0.961$ ) specifically for low concentrations of EtG in a subset of urine samples with EtG concentrations ranging from 100 to 5000 ng/mL (20). Intra- and interassay coefficients of variation were previously established at <1.7% and <2.2%, respectively (20). In accordance with previous research (21–23), a cutoff value of  $\geq 100$  ng/mL was defined for the assessment of alcohol consumption, indicating that EtG concentrations above this threshold were regarded as positive for intentional alcohol consumption. This cutoff value is highly sensitive. Controlled experiments have shown that EtG was already detectable after consumption of very small ethanol doses ( $\leq 10$  g) (24). Moreover, there are examples of unintentional alcohol “consumption” (e.g., alcohol in ethanol-based mouthwash) that could lead to low but detectable EtG concentrations in urine (25). To exclude these false-positive samples due to unintentional alcohol consumption, we included a sensitivity analysis in which we used a cutoff value of 500 ng/mL, which is also frequently used in the assessment of intentional alcohol consumption (14–16). We defined potential inadequate 24-h urine collections (i.e., over- or undercollection) as the upper and lower 2.5% of the difference between the estimated and measured volume of a subject’s 24-h urine sample. The estimated 24-h urine volume was derived from the following formula: creatinine clearance = [(urine creatinine  $\times$  24-h urine volume)/(serum creatinine)], where creatinine clearance was estimated using the Cockcroft–Gault formula.

#### Other biomarkers.

Plasma HDL cholesterol, erythrocyte mean corpuscular volume (MCV), and the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and  $\gamma$ -glutamyltransferase (GGT) were determined as indirect biomarkers of alcohol consumption using standard laboratory methods. Plasma AST, ALT, and GGT were measured by

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Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; EtG, ethyl glucuronide; FN, false negative; FP, false positive; GGT,  $\gamma$ -glutamyltransferase; MCV, erythrocyte mean corpuscular volume; PREVEND, Prevention of Renal and Vascular End-Stage Disease; TN, true negative; TP, true positive.

a standardized enzymatic method (Cobas c501; Roche Diagnostics). Serum HDL cholesterol was measured by a homogeneous method (direct HDL-C, Aerosep System; Abbott Laboratories), and MCV concentrations were measured using a Coulter Counter STKS sum (Coulter Corporation).

### Self-reported alcohol consumption

Participants were asked to complete a questionnaire regarding demographics, cardiovascular and renal history, drug use, alcohol consumption, and smoking. Participants were asked about the frequency of their habitual alcohol consumption with the following answer categories: 1) no, almost never; 2) 1–4 units/mo, 3) 2–7 units/wk, 4) 1–3 units/d, or 5)  $\geq 4$  units/d. In the Netherlands, a standard serving of an alcoholic beverage contains  $\sim 10$  g of alcohol. Alcohol consumption was defined according to the 5 categories of the questionnaire: abstinence, light, light-moderate, moderate, and heavy alcohol consumption.

### Other covariates

Information about age, sex, height, weight, educational level, and smoking was obtained from the questionnaire. Educational level was classified into the categories low (primary education or intermediate vocational education), middle (higher secondary education), and high (higher vocational education and university). Smoking status was categorized into nonsmoking, 1–10 cigarettes/d, 11–20 cigarettes/d, or  $> 20$  cigarettes/d. Estimated glomerular filtration rate (eGFR) was calculated using the combined creatinine cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation (26).

### Statistical analysis

All statistical analyses were performed using IBM SPSS 24.0 for Windows. We used descriptive statistics to assess the distribution of the data. Missing values were present for eGFR ( $n = 322$ , 5%), HDL cholesterol ( $n = 227$ , 3.7%), MCV ( $n = 44$ , 0.7%), AST ( $n = 220$ , 3.5%), ALT ( $n = 220$ , 3.5%), and GGT ( $n = 220$ , 3.5%) concentrations and replaced by the mean (mean eGFR =  $92.27$  mL/min/1.73 m<sup>2</sup>, mean HDL cholesterol =  $48.18$  mg/dL, and mean MCV =  $90.51$  fL,

respectively) or the median (median AST =  $23$  U/L, median ALAT =  $17$  U/L, and median GGT =  $24.0$  U/L, respectively).

We tabulated demographic and laboratory data stratified by the 5 alcohol consumption categories. We conducted the Pearson chi-square test to assess the correlation between EtG concentration and self-reported alcohol consumption. A contingency table was constructed, with EtG concentrations categorized into negative ( $< 100$  ng/mL) or positive ( $\geq 100$  ng/mL) for alcohol consumption. Self-reported alcohol consumption was categorized as “no alcohol consumption” or “alcohol consumption.” Alcohol consumption was further stratified into 4 categories. We calculated sensitivity [the number of true EtG positives (TPs) divided by the sum of TPs and false negatives (FNs)], specificity [the number of true EtG negatives (TNs) divided by the sum of TNs and false positives (FPs)], positive predictive value (TPs divided by the sum of TPs and FPs) and negative predictive value (TNs divided by the sum of TNs and FNs) of the EtG test for self-reported alcohol consumption categories.

For both self-report and urinary EtG, no established gold standards are available; therefore, the proportion of observed agreement was calculated. Agreement is an absolute measure because it quantifies the proportion of cases for which the 2 methods agree (27). In this analysis, agreement is the probability that a participant with a certain self-reported alcohol consumption has a corresponding urinary EtG concentration above or below cutoff. The proportion of specific agreement expresses the agreement separately for positive and negative ratings. In this case, the proportion of positive agreement is the proportion of participants who reported to be habitual alcohol consumers (i.e., who selected 1 of the 4 alcohol consumption categories) and have an EtG concentration  $\geq 100$  ng/mL. Subsequently, the proportion of negative agreement reflects the proportion of participants reporting abstinence who have a corresponding EtG concentration  $< 100$  ng/mL. Calculations of these proportions were based on the methods proposed by de Vet et al. (27). As a secondary analysis, all analyses were also performed using an EtG cutoff value of  $500$  ng/mL.

To test the continuous association between alcohol consumption categories and EtG concentration, we performed a multiple linear

**TABLE 1** Baseline characteristics of 6211 PREVEND participants<sup>1</sup>

	Alcohol consumption category (by self-report)					EtG concentration	
	No, almost never	1–4 units/mo	2–7 units/wk	1–3 units/d	$\geq 4$ units/d	$< 100$ ng/mL	$\geq 100$ ng/mL
Total participants, $n$ (%)	1539 (24.8)	1054 (17.0)	1969 (31.7)	1381 (22.2)	268 (4.3)	2997 (48.3)	3214 (51.7)
Men, $n$ (%)	599 (38.9)	487 (46.2)	1111 (56.4)	871 (63.1)	215 (52.9)	1389 (46.3)	1894 (58.9)
Women, $n$ (%)	940 (61.1)	567 (53.8)	858 (43.6)	510 (36.9)	53 (47.1)	1608 (53.7)	1320 (41.1)
Age, y	$55.7 \pm 12.6$	$53.9 \pm 13.0$	$51.5 \pm 11.6$	$54.1 \pm 11.0$	$54.6 \pm 9.9$	$54.3 \pm 12.7$	$53.1 \pm 11.4$
BMI, kg/m <sup>2</sup>	$26.9 [6.0]$	$26.3 [5.5]$	$25.9 [5.1]$	$25.7 [4.8]$	$26.3 [5.5]$	$26.7 [5.6]$	$25.7 [5.1]$
eGFR, mL/(min $\cdot$ 1.73 m <sup>2</sup> )	$88.6 \pm 18.3$	$91.1 \pm 16.7$	$94.5 \pm 15.7$	$93.7 \pm 15.4$	$93.9 \pm 15.0$	$90.5 \pm 17.3$	$94.0 \pm 15.7$
Smoking, $n$ (%)							
No smoking	551 (35.8)	379 (36.0)	579 (29.4)	250 (18.1)	29 (10.8)	1027 (34.3)	761 (23.7)
1–10 cigarettes/d	441 (28.7)	366 (34.7)	631 (32.0)	421 (30.5)	45 (16.8)	903 (30.1)	1001 (31.1)
11–20 cigarettes/d	396 (25.7)	246 (23.3)	573 (29.1)	502 (36.4)	109 (40.7)	790 (26.4)	1036 (32.2)
$> 20$ cigarettes/d	151 (9.8)	63 (6.0)	186 (9.4)	208 (15.1)	85 (31.7)	277 (9.2)	416 (12.9)
Educational level, $n$ (%)							
Low	942 (61.2)	479 (45.4)	684 (34.7)	478 (34.6)	112 (41.8)	1554 (51.9)	1141 (35.5)
Middle	379 (24.6)	288 (27.3)	534 (27.1)	332 (24.0)	65 (24.3)	760 (25.4)	838 (26.1)
High	218 (14.2)	287 (27.2)	751 (38.1)	571 (41.3)	91 (34.0)	683 (22.8)	1235 (38.4)
Plasma analytes							
HDL-C, mg/dL	$46.3 \pm 10.9$	$47.3 \pm 10.7$	$48.3 \pm 11.5$	$50.2 \pm 12.3$	$50.7 \pm 13.2$	$46.6 \pm 10.8$	$49.7 \pm 12.1$
MCV, fL	$89.4 \pm 4.7$	$89.6 \pm 4.5$	$90.5 \pm 4.2$	$91.7 \pm 4.4$	$94.0 \pm 4.7$	$89.7 \pm 4.6$	$91.3 \pm 4.5$
AST, U/L	$22.0 [7.0]$	$22.0 [6.0]$	$23.0 [7.0]$	$23.0 [7.0]$	$25.0 [8.0]$	$22.0 [7.0]$	$23.0 [7.0]$
ALT, U/L	$17.0 [11.0]$	$17.0 [10.0]$	$17.0 [11.0]$	$18.0 [11.0]$	$20.0 [14.5]$	$17.0 [11.0]$	$17.0 [12.0]$
GGT, U/L	$24.0 [18.0]$	$22.0 [18.0]$	$24.0 [21.0]$	$27.0 [25.0]$	$37.5 [46.8]$	$24.0 [19.0]$	$25.0 [24.0]$

<sup>1</sup>Values are  $n$  (%), means  $\pm$  SDs, or medians [IQRs]. Educational level: low, primary education or intermediate vocational education; middle, higher secondary education; and high, higher vocational education and university. ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; EtG, ethyl glucuronide; GGT,  $\gamma$ -glutamyltransferase; HDL-C, HDL cholesterol; MCV, mean corpuscular volume; PREVEND, Prevention of Renal and Vascular End-Stage Disease.



regression analysis. Because of the skewed distribution, the natural logarithm of EtG concentration was used as continuous outcome. We included only participants with detectable EtG concentrations (i.e., concentrations  $\geq 100$  ng/mL) in this analysis ( $n = 3219$ ). The “abstention” alcohol category was used as reference. First, a crude analysis (model 1) was conducted. Age, sex, and eGFR are potential effect modifiers for EtG concentrations (21–23). Therefore, these variables and their interaction terms with alcohol consumption were separately entered in the model to test for influence on the relation between EtG concentration and alcohol consumption (model 2). Age was added as a continuous variable, whereas eGFR was added as a categorical variable, defined as either impaired renal function [eGFR  $< 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>)] or normal renal function [eGFR  $\geq 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>)]. In case significant interaction terms ( $P < 0.10$ ) in our minimally adjusted model were found, analyses were stratified accordingly. Data of these linear regression analyses are presented as geometric means. To study the association between indirect biomarkers and EtG concentrations, we performed a second regression analysis. Data of this analysis are presented as back-transformed coefficients that indicate the increase in EtG concentration per 1 unit increase in biomarker concentration. As a sensitivity analysis, we reanalyzed the data excluding subjects with potential inadequate 24-h urine collections. Furthermore, we repeated the regression analyses excluding the participants with missing data to assess the effect of imputation on our results.

## Results

### Participant characteristics

Among 6211 PREVENT participants, the mean age was 53.7 y (SD = 12.0), and 52.9% were men. Of the participants, 75.2% consumed alcohol according to self-report (Table 1). Women reported lower alcohol consumption compared with men and were more often abstainers (32% compared with 18%, respectively). Moderate to heavy drinkers were more often current smokers: 64% of the participants in the abstention group smoked at least 1 cigarette a day compared with 82% in the moderate alcohol consumption group and 89% in the heavy alcohol consumption group.

### Detection of EtG

In 51.7% of the study population, EtG concentrations were above detection limit ( $\geq 100$  ng/mL), indicating intentional alcohol consumption. Cross-tabulations (Table 2) between self-reported alcohol consumption and EtG concentration indicate that 92.3% (95% CI: 91.6%, 93.0%) of the participants with self-reported abstention had an EtG concentration  $< 100$  ng/mL. On the other hand, 33.7% (95% CI: 33.1%,

34.3%) of the participants with self-reported alcohol consumption had a discordant EtG concentration below cutoff value. Sensitivity for EtG as a marker is 66.3% (95% CI: 65.1%, 67.5%). When stratified for alcohol consumption category, sensitivity became higher for higher alcohol consumption: 30.3% (95% CI: 29.2%, 31.4%) in category 1–4 units/mo, 64.2% (95% CI: 63.0%, 65.4%) in category 2–7 units/wk, 91.5% (95% CI: 90.8%, 92.2%) in category 1–3 units/d, and 92.5% (95% CI: 91.8%, 93.2%) in category  $\geq 4$  units/d. The positive predictive value was 96.3%, and the negative predictive value was 47.4%.

### Measure of agreement

Proportion of positive agreement, referring to the proportion of participants indicating alcohol consumption on self-report that also had an EtG concentration  $\geq 100$  ng/mL, was 78.5% (95% CI: 77.4%, 79.5%). Proportion of negative agreement, reflecting the proportion of participants reporting abstention with a corresponding EtG concentration  $< 100$  ng/mL, was 62.7% (95% CI: 61.5%, 63.9%). In the multiple linear regression, the interaction term for eGFR was statistically significant ( $P = 0.01$ ). Therefore, we also calculated agreement stratified for eGFR. Proportion of positive agreement was lower, whereas proportion of negative agreement was higher, in participants with an impaired renal function [eGFR  $< 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>)] compared with participants with a normal renal function [eGFR  $\geq 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>)] (Table 3).

### Sensitivity analyses

When a higher cutoff value for EtG was used (i.e., 500 ng/mL), the diagnostic measures were consistently lower (Table 3), except for specificity and positive predictive value. Exclusion of subjects with potential inadequate 24-h urine collection did not alter any of the results (data not shown).

### Association between self-reported alcohol consumption and EtG concentrations

EtG concentrations were directly associated with categories of increasing alcohol consumption ( $P < 0.001$ ) (Table 4). Because the interaction term for eGFR showed a significant interaction ( $P$ -interaction = 0.01), the regression analyses were also stratified for eGFR [eGFR  $< 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>) and eGFR  $\geq 90$  mL/(min  $\cdot$  1.73 m<sup>2</sup>)]. However, stratified analyses did not show different results for renal function categories. Exclusion of participants with missing data for eGFR ( $n = 169$ ) did not alter the results (data not shown).

**TABLE 2** Cross-tabulation between self-reported alcohol consumption and urinary EtG concentration among 6211 PREVENT participants using a cutoff value of 100 ng/mL<sup>1</sup>

	Interpretation of EtG concentration		
	Positive ( $\geq 100$ ng/mL)	Negative ( $< 100$ ng/mL)	Total
Self-reported alcohol consumption, $n$ (%)			
No	118 (7.7)	1421 (92.3)	1539
Yes	3096 (66.3)	1576 (33.7)	4672
Stratified by alcohol consumption category, $n$ (%)			
1–4 units/mo	319 (30.3)	735 (69.7)	1054
2–7 units/wk	1265 (64.2)	704 (35.8)	1969
1–3 units/d	1264 (91.5)	117 (8.5)	1381
$\geq 4$ units/d	248 (92.5)	20 (7.5)	268
Total, $n$ (%)	3214 (51.7)	2997 (48.3)	6211

<sup>1</sup> EtG, ethyl glucuronide; PREVENT, Prevention of Renal and Vascular End-Stage Disease.

**TABLE 3** Diagnostic measures for EtG cutoff values of 100 and 500 ng/mL in 6211 PREVEND participants<sup>1</sup>

	Cutoff: 100 ng/mL (%)	Cutoff: 500 ng/mL (%)
Total ( <i>n</i> = 6211)		
Sensitivity	66.3	57.1
Specificity	92.3	96.0
Positive predictive value	96.3	97.8
Negative predictive value	47.4	42.5
Proportion positive agreement	78.5	72.1
Proportion negative agreement	62.7	58.9
Impaired renal function [eGFR <90 mL/(min · 1.73 m <sup>2</sup> )] ( <i>n</i> = 2425)		
Proportion positive agreement	75.6	68.7
Proportion negative agreement	66.4	63.1
Normal renal function [eGFR ≥90 mL/(min · 1.73 m <sup>2</sup> )] ( <i>n</i> = 3786)		
Proportion positive agreement	80.1	74.0
Proportion negative agreement	59.6	55.5

<sup>1</sup>Comparison of diagnostic measures for different cutoff values of EtG concentrations. Sensitivity: TP/(TP + FN); specificity: TN/(TN + FP); positive predictive value: TP/(TP + FP); negative predictive value: TN/(TN + FN). eGFR, estimated glomerular filtration rate; EtG, ethyl glucuronide; FN, false EtG negatives; FP, false EtG positives; PREVEND, Prevention of Renal and Vascular End-Stage Disease; TN, true EtG negatives; TP, true EtG positives.

### Association between indirect biomarkers and EtG concentrations

Linear regression analyses showed a significant positive association between HDL cholesterol (unstandardized  $\beta$ : 1.01 ng/mL; 95% CI: 1.01, 1.02 ng/mL), MCV ( $\beta$ : 1.10 ng/mL; 95% CI: 1.08, 1.11 ng/mL), and GGT biomarkers ( $\beta$ : 1.01 ng/mL; 95% CI: 1.00, 1.01 ng/mL) and EtG concentrations. Plasma AST ( $\beta$ : 1.00 ng/mL; 95% CI: 0.99, 1.01 ng/mL) and ALT ( $\beta$ : 1.00 ng/mL; 95% CI: 0.99, 1.01 ng/mL) were not significantly associated with EtG concentrations. Adjustment for age, sex, and eGFR did not alter these results, neither did exclusion of participants with missing values (*n* = 219) (data not shown).

### Discussion

In this study, we showed that EtG is in reasonable agreement with self-report when assessing habitual alcohol consumption. The positive predictive value was 96%, the negative predictive value was 48%, and specificity was high (92%). By contrast, overall sensitivity was only 66.3%, which increased in the moderate to heavy drinking categories (91–93%). Furthermore, the proportions of positive and negative agreement were 78.5% and 62.7%, respectively, both reflecting a reasonable agreement between urinary EtG and self-reported alcohol consumption.

Categories of increasing alcohol consumption were linearly associated with EtG concentrations. Moreover, some, but not all, indirect biomarkers of alcohol consumption were linearly associated with EtG concentrations.

### Strengths and limitations

Our study is the first to compare urinary EtG concentrations to self-reported habitual alcohol consumption and other biomarkers in a large cohort. Until now, measurements of urinary EtG concentrations on such a large scale have not been performed in epidemiologic research. Because of the large sample size, we were able to calculate precise estimates of sensitivity, specificity, and positive and negative predictive value of urinary EtG as a diagnostic test. Moreover, our study is the first to compare urinary EtG concentrations with both self-reported consumption and other biomarkers of alcohol consumption. However, our study has some limitations.

It is important to acknowledge that no gold standard for habitual alcohol consumption is available. We used self-reported consumption to estimate the predictive value of EtG as a biomarker. Therefore, measurement error in self-report could have affected the estimated accuracy of EtG.

Furthermore, we based our self-reported alcohol consumption data on a questionnaire that provided only limited information. Habitual alcohol consumption was divided into

**TABLE 4** Associations between alcohol consumption categories and EtG concentrations in PREVEND participants with detectable EtG concentrations (*n* = 3219)<sup>1</sup>

	Alcohol consumption category					<i>P</i> -trend
	Abstinence (reference)					
	( <i>n</i> = 119)	Light drinkers ( <i>n</i> = 319)	Light-moderate drinkers ( <i>n</i> = 1268)	Moderate drinkers ( <i>n</i> = 1265)	Heavy drinkers ( <i>n</i> = 248)	
Model 1 <sup>2</sup>	620 (470, 820)	1068 (583, 1955)	2343 (1326, 4138)	5497 (3109, 9711)	15,139 (8168, 28,057)	<0.001
Model 2 <sup>3</sup>	670 (409, 1097)	1064 (467, 2426)	2109 (935, 4755)	4346 (1850, 10,209)	10,467 (4081, 26,849)	<0.001

<sup>1</sup>All values are geometric means (95% CIs) of linear regression analyses and were found to be statistically significant, *P* < 0.001. eGFR, estimated glomerular filtration rate; EtG, ethyl glucuronide; PREVEND, Prevention of Renal and Vascular End-Stage Disease.

<sup>2</sup>Crude analysis.

<sup>3</sup>Adjusted for age (y), sex, and eGFR [mL/(min · 1.73 m<sup>2</sup>)].

5 categories, and the time since last alcohol consumption was not recorded. Therefore, only the global distinction between abstinent, light, light-moderate, moderate, and heavy drinkers could be made. It was not possible to relate EtG concentrations with fixed amounts of alcohol; therefore, it remains unclear whether the concentrations of EtG can reflect certain amounts of alcohol at an individual level. Nevertheless, we have shown that on a group level, EtG concentrations increase with increasing alcohol consumption categories. Therefore, the results from our study can be used to draw conclusions on a group level but not on an individual level.

Another limitation is the use of the natural logarithm of EtG concentration as a dependent variable in the multiple linear regression analysis. Using a log-transformed variable leads to the loss of cases for which the EtG concentration was below the detection limit and therefore to an overestimation of EtG concentrations in the “abstention” category. The majority of the cases in the abstention category in this analysis will consist of false positives, leading to an underestimation of the true differences between consumption categories.

### Explanation of results

In our study, we found that sensitivity was low compared with that of previous studies, in which sensitivity ranged from 79% to 100% (11–16). This is probably because we studied *habitual* alcohol consumption, which ranges from abstention to more than 4 units per day. EtG is only detectable up to 72 h after alcohol consumption. Consequently, a substantial part of the group of light alcohol consumers (1–4 units/mo) will have an EtG concentration below the cutoff value, leading to misclassification of these consumers. The categories in which participants drank at least 1 alcoholic consumption per day (the moderate and heavy consumption categories) had a higher sensitivity (91–93%). Repeated sampling of urine on different days, also taking into account the differences in alcohol consumption patterns during weekdays and weekends, would reduce the misclassification of light drinkers because this increases the chance of detecting EtG in the people who consume alcohol only occasionally. For convenience, EtG can be measured in spot urine samples if corrected for urinary creatinine concentration to compensate for urine dilution (28). Repeated urine sampling, however, might be a less feasible option in large-scale population research.

The reasonable proportions of agreement between EtG and self-reported alcohol consumption do not entirely reflect the concerns of underreporting of alcohol consumption as raised in previous research, in which urinary EtG concentrations were compared to self-reported alcohol consumption in more controlled settings. In these studies, discordance between EtG and self-reported abstention was up to 40% (16). In our study, only 8% of the participants who reported abstention had positive EtG concentrations. The discrepancy can probably be explained by differences in study population and study design: These studies used a population of alcohol-dependent persons in an abstention program, whereas our study population was a sample of the general population, in which the focus was not on alcohol consumption. Compared to the general population, alcohol-dependent persons in an abstention program are probably more likely to underreport alcohol consumption because this might have consequences for their participation in the program. Furthermore, urine testing will probably be carried out at a time when the chance of catching unallowed alcohol consumption is highest.

Proportions of agreement differed when stratified by eGFR. Participants with impaired renal function had higher proportions of negative agreement and lower proportions of positive agreement. This can potentially be explained by the fact that proportion of agreement is related to prevalence of, in this case, alcohol consumption (27). People with impaired renal function are more often abstainers compared with people with a normal renal function, probably because of health-related issues.

We found an association between urinary EtG and the indirect biomarkers HDL cholesterol, MCV, and GGT but not with AST and ALT. These findings are in line with the results of Kilo et al. (17), who also examined habitual alcohol consumption. This consistently demonstrates that some, but not all, indirect biomarkers are associated with EtG concentrations in a general population with a wide range of alcohol consumption—from abstainers to heavy drinkers.

The utility of the application of EtG as a biomarker for alcohol consumption is highly dependent on the cutoff value used. Cutoff values most often used are 100, 200, and 500 ng/mL. Previous studies have commented on the limitations of each cutoff value (11, 22). In the current study, the motivation to choose 100 ng/mL as a cutoff value was largely based on previous studies that posed a research question similar to ours (13, 21, 22). McDonnell et al. (13) calculated the sensitivity and specificity for cutoff values of 100, 200, and 500 ng/mL in light and heavy drinking in alcohol-dependent patients following an abstention program. They concluded that a cutoff value of 100 ng/mL was likely to detect both light and heavy drinkers, whereas a cutoff value of 500 ng/mL could only detect the heavy drinkers during the previous day. Using a low cutoff value can lead to more false-positive cases. A cutoff value of 500 ng/mL would exclude all false-positive cases, but it would lead to lower sensitivity, especially in light to moderate drinkers.

The choice of the “right” cutoff value remains subject to debate. However, when applying a cutoff value of 500 ng/mL in our study, similar conclusions can be drawn. Specificity and positive predictive value increase at the cost of a decrease in sensitivity, most pronounced in the lower alcohol consumption categories.

In conclusion, this study shows that urinary EtG can be used as a measure of habitual alcohol consumption on a group level in epidemiologic research. The advantage of using an objective biomarker instead of a subjective approach for alcohol consumption is that it is not hampered by recall error or misclassification. Future studies should include the use of urinary EtG in the assessment of alcohol as a risk factor for chronic diseases in population research.

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### References

1. Rehm J, Taylor B, Mohapatra S, Irving H, Baliunas D, Patra J, Roerecke M. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. *Drug Alcohol Rev* 2010;29(4):437–45.
2. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ* 2011;342:d671.



3. Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* 1998;279(7):535–40.
4. Stockwell T, Donath S, Cooper-Stanbury M, Chikritzhs T, Catalano P, Mateo C. Under-reporting of alcohol consumption in household surveys: a comparison of quantity-frequency, graduated-frequency and recent recall. *Addiction* 2004;99(8):1024–33.
5. Savola O, Niemela O, Hillbom M. Blood alcohol is the best indicator of hazardous alcohol drinking in young adults and working-age patients with trauma. *Alcohol Alcohol* 2004;39(4):340–5.
6. Ingall GB. Alcohol biomarkers. *Clin Lab Med* 2012;32(3):391–406.
7. Niemela O. Biomarkers in alcoholism. *Clin Chim Acta* 2007;377(1–2):39–49.
8. Walsham NE, Sherwood RA. Ethyl glucuronide. *Ann Clin Biochem* 2012;49(Pt 2):110–7.
9. Wurst FM, Metzger J, WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators. The ethanol conjugate ethyl glucuronide is a useful marker of recent alcohol consumption. *Alcohol Clin Exp Res* 2002;26(7):1114–9.
10. Kissack JC, Bishop J, Roper AL. Ethylglucuronide as a biomarker for ethanol detection. *Pharmacotherapy* 2008;28(6):769–81.
11. Armer JM, Gunawardana L, Allcock RL. The performance of alcohol markers including ethyl glucuronide and ethyl sulphate to detect alcohol use in clients in a community alcohol treatment programme. *Alcohol Alcohol* 2017;52(1):29–34.
12. McDonell MG, Leickly E, McPherson S, Skalsky J, Srebnik D, Angelo F, Vilardaga R, Nepom JR, Roll JM, Ries RK. A randomized controlled trial of ethyl glucuronide-based contingency management for outpatients with co-occurring alcohol use disorders and serious mental illness. *Am J Psychiatry* 2017;174(4):370–7.
13. McDonell MG, Skalsky J, Leickly E, McPherson S, Battalio S, Nepom JR, Srebnik D, Roll J, Ries RK. Using ethyl glucuronide in urine to detect light and heavy drinking in alcohol dependent outpatients. *Drug Alcohol Depend* 2015;157:184–7.
14. Wetterling T, Dibbelt L, Wetterling G, Goder R, Wurst F, Margraf M, Junghanns K. Ethyl glucuronide (EtG): better than breathalyzer or self-reports to detect covert short-term relapses into drinking. *Alcohol Alcohol* 2014;49(1):51–4.
15. Helander A, Bottcher M, Fehr C, Dahmen N, Beck O. Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. *Alcohol Alcohol* 2009;44(1):55–61.
16. Barrio P, Teixidor L, Rico N, Bruguera P, Ortega L, Bedini JL, Gual A. Urine ethyl glucuronide unraveling the reality of abstinence monitoring in a routine outpatient setting: a cross-sectional comparison with ethanol, self report and clinical judgment. *Eur Addict Res* 2016;22(5):243–8.
17. Kilo S, Hofmann B, Eckert E, Goen T, Drexler H. Evaluation of biomarkers assessing regular alcohol consumption in an occupational setting. *Int Arch Occup Environ Health* 2016;89(8):1193–203.
18. Pinto-Sietsma SJ, Janssen WM, Hillege HL, Navis G, De Zeeuw D, De Jong PE. Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. *J Am Soc Nephrol* 2000;11(10):1882–8.
19. Helander A, Dahl H. Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. *Clin Chem* 2005;51(9):1728–30.
20. Bottcher M, Beck O, Helander A. Evaluation of a new immunoassay for urinary ethyl glucuronide testing. *Alcohol Alcohol* 2008;43(1):46–8.
21. Ferraguti G, Ciolli P, Carito V, Battagliese G, Mancinelli R, Ciafre S, Tirassa P, Ciccarelli R, Cipriani A, Messina MP. Ethylglucuronide in the urine as a marker of alcohol consumption during pregnancy: comparison with four alcohol screening questionnaires. *Toxicol Lett* 2017;275:49–56.
22. Jatlow PI, Agro A, Wu R, Nadim H, Toll BA, Ralevski E, Nogueira C, Shi J, Dziura JD, Petrakis IL. Ethyl glucuronide and ethyl sulfate assays in clinical trials, interpretation, and limitations: results of a dose ranging alcohol challenge study and 2 clinical trials. *Alcohol Clin Exp Res* 2014;38(7):2056–65.
23. Wurst FM, Wiesbeck GA, Metzger JW, Weinmann W. On sensitivity, specificity, and the influence of various parameters on ethyl glucuronide levels in urine—results from the WHO/ISBRA study. *Alcohol Clin Exp Res* 2004;28(8):1220–8.
24. Stephanson N, Dahl H, Helander A, Beck O. Direct quantification of ethyl glucuronide in clinical urine samples by liquid chromatography–mass spectrometry. *Ther Drug Monit* 2002;24(5):645–51.
25. Costantino A, Digregorio EJ, Korn W, Spayd S, Rieders F. The effect of the use of mouthwash on ethylglucuronide concentrations in urine. *J Anal Toxicol* 2006;30(9):659–62.
26. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YP, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012;367(1):20–9.
27. de Vet HC, Mokkink LB, Terwee CB, Hoekstra OS, Knol DL. Clinicians are right not to like Cohen's kappa. *BMJ* 2013;346:f2125.
28. Dahl H, Stephanson N, Beck O, Helander A. Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. *J Anal Toxicol* 2002;26(4):201–4.